Mass rearing the endangered Palos Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis:* Lycaenidae)

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Abstract: Mass rearing of the endangered lycaenid *Glaucopsyche lygdamus palosverdesensis* (Palos Verdes blue butterfly) is described. Numerous problems were encountered in our attempts to predictably produce a large stock population both as insurance against extinction and for re-introduction to sites where the species has been extirpated. We describe our approaches to mass rearing with discussion of all aspects of life history, difficulties with parasitoids and predators, cage design, and artificial diet use. Both cylindrical cages placed over individual potted plants and outdoor tent cages were successful in providing conditions where captive individuals would mate without intervention, transcending previous limits posed by hand pairing. From a small initial stock, we produced between 168 and 968 pupae each season. Highest losses were experienced in first instar, with later losses from microsporidian infection. Predation during pupation was also significant in semi-natural confined conditions. The effort has been in progress for eight years and is continuing.

Key Words: *Glaucopsyche lygdamus palosverdesensis*, Palos Verdes blue butterfly, mass rearing, artificial diet, mass selection, predators and parasitoids, cage design.

Introduction

The scientific literature reports few attempts to mass rear butterflies, that is, to produce large quantities of a species for experimental, or, more recently, conservation purposes (Mattoon et al. 1971; Lees 1989; Herms et al. 1996). Production under less controlled conditions has been explored through butterfly ranching as a tool for conservation and sustainable harvest of tropical butterfly species (Parsons 1984; New 1994). Methods for mass rearing of butterflies in more controlled conditions have not been thoroughly described, notwithstanding well developed methods to mass produce several moth species for economic purposes as sterile control programs, and success producing many insect parasitoids for biocontrol (Parrella et al. 1992; Hassan 1993). In the latter cases production of millions of individuals per day have been achieved (King & Leppla 1984; Thompson 1999). Development of artificial diets allowed these production levels (Singh & Moore 1985; Anderson & Leppla 1992), but only for species that mate rapidly in confined spaces.

Because life histories of many butterfly species are relatively well known, mass rearing would seem relatively simple given adequate funding resources. However, because very few butterflies have recognized economic value, few incentives exist to develop such methodology (Lees 1989; Samways 1990). With advent of the U.S. Endangered Species Act, captive pro-pagation and mass rearing may now be heuristic endeavors. Already many programs have been imple-mented, several at the cost of many millions of dollars, to rescue nearly extinct animal species. Conservation agencies devoted extensive resources to captive rearing of vertebrates, most famously California condor and black-footed ferret (Meffe & Carroll 1997), while some listed butterflies have also been reared in captivity (Herms et al. 1996).

Although all butterflies are amenable to captive rearing, including the usually difficult problem of inducing mating, large scale production is not in place. Butterfly farming for butterfly houses, production of specimens for release at special events, and educational use for hands-on student observation of metamorphosis has increased (New 1994). Although there are no quantitative estimates of production rates, these are labor intensive and fall far short of constituting an industrial, predictive process.

All groups of butterflies have been captive reared, at least from egg to adult, with most efforts depending upon natural foodplants. The limiting factor to continuous rearing of many species has been inducement of mating, for which hand pairing was developed (Clarke & Sheppard 1956). The technique is tedious, impractical for mass rearing, and likely results in unwanted artificial selection. Below we describe methods that breach the limits of hand pairing for an endangered butterfly species, the Palos Verdes blue butterfly (*Glaucopsyche lygdamus palosverdesensis*).

Following rediscovery of the Palos Verdes blue butterfly in 1994 at the Defense Fuel Support Point (DFSP), San Pedro, California, a captive propagation effort was begun (Mattoni 1994). It was immediately apparent that this sole population of the species was in danger of extinction from stochastic factors; the wild population was only a few hundred (Mattoni 1994). The rearing program has operated since 1995, and this paper outlines the methods and results for captive rearing through the 2002 season. Unless specifically stated, the techniques used, results, and problems were from 2002. The rearing project has been conducted with a permit from the U.S. Fish and Wildlife Service (USFWS; Mattoni: TE-807303-4). As such, the program initially followed methods recommended by the USFWS for endangered lycaenid butterflies that previously had been developed by Mattoni (1988).

The three objectives of the captive breeding program for the Palos Verdes blue butterfly were: 1) to provide insurance against stochastic loss of the sole and diminished population of this species; 2) to increase size of this only known population of the insect at DFSP and; 3) to produce sufficient numbers of individuals to reintroduce the species onto revegetated sites from which it has been extirpated across the Palos Verdes peninsula. Thus far, the program has achieved all three goals — we have maintained a captive population since 1995, we established new populations of the butterfly from captive stock at DFSP, and we attempted a reintroduction in the former range of the species with captive reared stock.

This paper reports on the rearing process itself, details about reintroductions on and off the DFSP site are reported elsewhere (Mattoni 2002).

Genetic Considerations

Mass selection

Under any breeding system changes in gene frequency will occur across generations by either natural or artificial selection, or random sampling (genetic drift) (Mackauer 1972; Mackauer 1976). The changes are inevitable because the environment of the breeding system will not be the same as the environment of the natural habitat. Both pre- and post-zygotic selection will occur whether detectable or not. If the breeding system is designed to save and randomly mate every individual, at some point more individuals are produced than resources can maintain. The goal of any captive breeding system for conservation is to retain the substantial hidden genetic variation within natural populations (see Dimock & Mattoni 1986), and to reduce drift and selection on the population so that the resulting individuals maintain their adaptation to natural conditions (see Nunney 2002).

The captive propagation program then must establish the end use of stocks, a decision that must be taken in view of the relationship of N_e of the natural population and its ecological and genetic circumstances. Questions to be considered are whether the captive population should be maintained in parallel using only the original captures, whether new wild stock be introduced into the captive stock, or whether there be regular releases of captives while simultaneously introducing new wilds, or not, into the captive stock. Under any scenario, however, ease of consistent production of large numbers of individuals remains the key consideration. Until this objective is reached — and it has not been — other issues are moot.

We refer to mass rearing simply as the production of large numbers of individuals from a small initial

stock, then expanded by randomly mating all offspring of the following generations. Mass selection refers to the emphasis on random mating. This does not imply that selection is not occurring, but rather that as little as possible influence is exerted on the choice of mates within the system, or on the survival of any given individual. Accordingly as adults eclose, they are accumulated for one to two days and then either mated as two pairs set into small (gallon) cages, or more than two pairs into large (tent) cages. If fertile, eggs are laid on foodplant and larvae allowed to develop. The concept is that some choice of mate is provided and all offspring are given equal opportunity to develop. Thus selection is a mass phenomenon with minimal manipulative intrusion.

Others have suggested that naturalistic conditions may be used to reduce the selective effect of laboratory conditions on captive stock of insects (Boller 1972; Mackauer 1976). The use of outdoor tents is consistent with this suggestion, and is important within a conservation context where it is essential that the reared stock retain its adaptation to natural conditions (Mackauer 1976). Bryant and others (Bryant et al. 1999) by contrast emphasize the maintenance of fitness in captive populations by selectively mating high performance breeders or by high frequency immigration. Whatever approach is taken must depend on overall management goals and objectives. We believe mass selection combined with periodic immigration of wild stock is preferable at DFSP.

Breeding Stock

The adult stock for 2002 was almost entirely derived from progeny of five wild females originally confined in 1999. The only new genetic resources were 12 pupae from four wild females taken and confined in 2000. The six adults that eclosed from the wild stock were randomly mixed with 692 year 2000 adult offspring used for the 2001 breeding population. The resultant 2001 pupal population in turn produced 150 adults. These were combined with 17 adults from the carryover pupae from 2000 for the 2002 breeding stock.

Indeed every captive adult was involved in the breeding system after year 1999. Although most production was from a few cages (e.g., of the 150 adults from 2001, 72% were derived from two tent cages) this clustering may not have had a bottleneck effect given the small initial stock. Even assuming operation of some selection process, the likelihood of increases in homozygosity and/or loss of alleles cannot be significant given the numbers produced in 2002 (165) relative to the original five females from 1999.

Materials

After experimenting with a miscellany of cage configurations, we adopted two types of confinement chambers for general use after year 2000. The first, "gallon cages," consisted of either a cylinder of clear vinyl plastic or standard 16" x 18" mesh metal window screen fashioned to fit within the rim of a standard 6" one gallon, plastic nursery pot (Fig. 1). Foodplant was propagated in the pots. The cylinders were 12 to 18 inches tall to contain the foodplants. The cages were used both for mating and subsequent rearing of larvae.

The second were "tent" cages, consisting of 0.75 inch white PVC tubing joined with standard fittings to form approximately 4 foot square by 3 foot tall frameworks which were covered with flexible plastic window screen. The tops, or roofs, were affixed using Velcro strips to facilitate access to the cage interiors. The tent corner posts were driven into the ground and the bottom edge of the screens buried to prevent loss of adults (Fig. 2). The tent cages were placed over closely planted clumps or individual large foodplant specimens in the field. Tops werenecessary because rain can collect on exposed screens to create drops large enough to drown adults incages. This was necessitated because of water pooling on the screen top with concentration and drowning of adults present.

Maintenance of adult viability in the gallon cages depended on regular feeding of a 20% honey in water solution once daily for about one hour. Earlier *ad libidum* feeding led to bloat and early death. Tent cages were placed over large foodplants with abundant flowers, which provided a sufficient natural nectar source.

Larvae were usually removed from both cage types at various stages as discussed below. These larvae were maintained individually in one ounce polystyrene cups (creamers) using either foodplant pieces or artificial diet. Pupation took place in the cups.

Fig. 1

Fig. 2

Fig. 1. Gallon cage for controlled rearing of Palos Verdes blue butterfly.
Fig. 2. Tent cage for naturalistic rearing environment for Palos Verdes blue butterfly.
Fig. 5. Screen cylinder to allow eclosion and wing expansion of captive butterflies.
Fig. 6. Failed wing expansion in Palos Verdes blue butterfly.
Fig. 7. Aberrant Palos Verdes blue butterfly with reduced underside secondary macules.
Fig. 8. Aberrant Palos Verdes blue butterfly with exaggerated postmedial underside macules.
Fig. 9. Palos Verdes blue butterfly larvae with distended prothoracic segments.
Fig. 10. Male Palos Verdes blue butterfly caught in spider web.

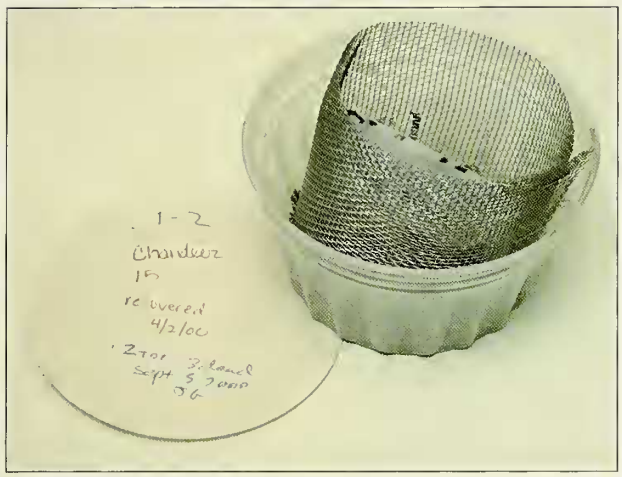

Fig. 5

Fig. 6

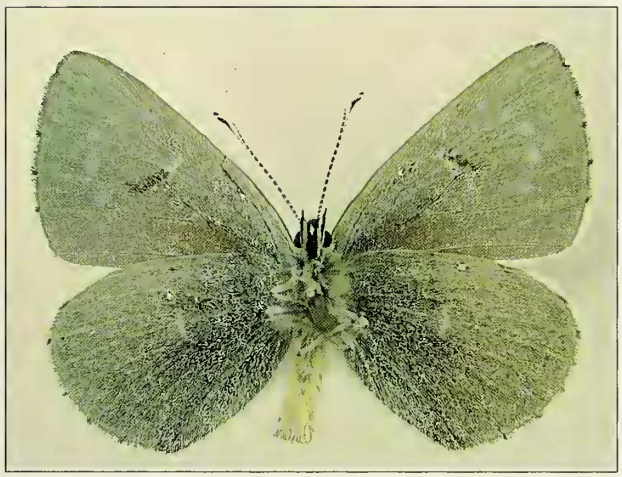

Fig. 7

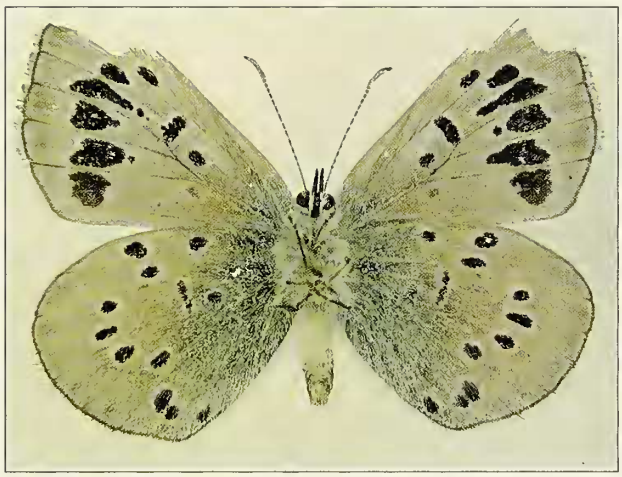

Fig. 8

Fig. 9

Fig. 10

Rearing Methods and Life History Characteristics

Pupae and Diapause

The pupae diapause under refrigeration and are synchronized for eclosion under continuous cold. We determined that eclosion occurs about two weeks following removal from cold to ambient temperature (~20 °C). In 2002 pupae were removed on February 24 and began eclosion March 8, the last adult emerging March 16. Time from removal from refrigeration to eclosion was normally distributed with a mean of 16 days (Fig. 3). Of 342 possible viable pupae from 2000 and 2001, 165 (48%) eclosed. Although many of the non-eclosed pupae were probably not viable, some fraction remained in diapause. Left under ambient conditions without refrigeration, eclosion can extend over a period of at least six weeks. Refrigeration is therefore a useful technique to synchronize eclosion to facilitate mating in the captive rearing setting.

Immediately following removal from refrigeration, all pupae were weighed to estimate how many were viable. A frequency distribution of all pupal weights after diapause showed a distinct bimodal distribution (Fig. 4), while pupae before diapause show a normal distribution (Longcore et al. 2002). After diapause, weights of pupae less than 50 mg formed a normal distribution (skewness = 0.19; mean weight = 27.2 ± 10.0 s.d.), and weights of pupae greater than 50 mg formed a normal distribution (skewness = –0.10; mean weight = 86.1 ± 17.2 s.d.). Pupae less than 50 mg were assumed to be not viable, while pupae greater than 50 mg were assumed to be potentially viable. The hypothesis that 50 mg indicates a cutoff for viability was partially confirmed by the pattern of eclosion. No pupae below 50 mg produced adults, while 48% of those presumed viable did.

Eclosion

Adults from fourteen pupae were unable to escape the pupal cases or failed to expand their wings. Most, if not all, were failures due to faulty physical environmental condition, e.g. positioning of the pupae that prevented their normally grasping a structure that would provide leverage to crawl from the pupal case. Although such failures must occur in nature, our artificial system is likely flawed. Fig. 5 illustrates the screen cylinders within which pupae are placed for eclosion. Emerding adults can climb the screen wall to allow full wing expansion. Fig. 6 illustrates an individual with failed wing expansion.

Mating

Mating lycaenid butterflies in captivity has a variable success rate. Hoegh-Guldberg (1979)

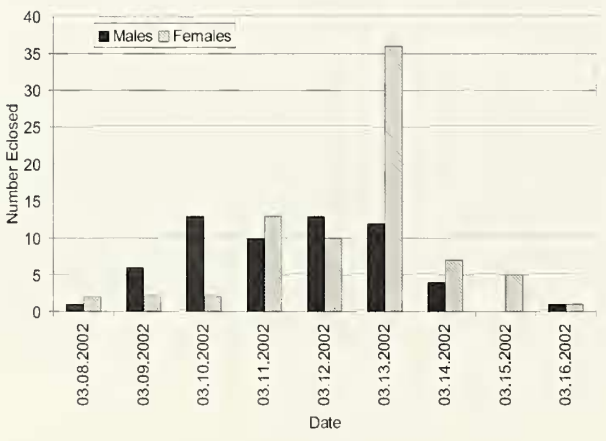

Fig. 3. Eclosion of adult Palos Verdes blue butterflies under laboratory conditions. Pupae were removed from refrigeration on February 24, 2002.

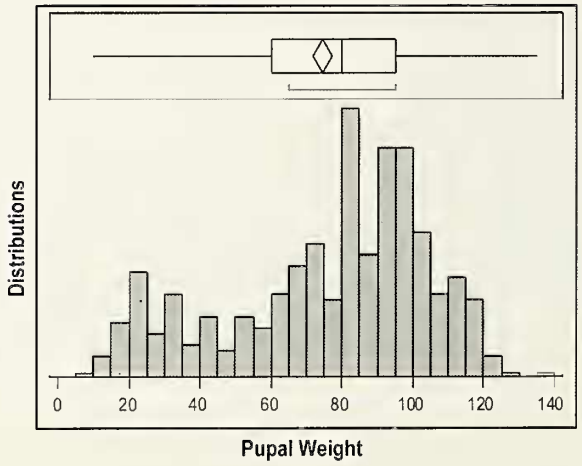

Fig. 4. Distribution of Palos Verdes blue butterfly pupae weights in mg when removed from refrigeration to break diapause. Note bimodal distribution with peaks at 90–100 mg and 20–30 mg (skewness = –0.64).

successfully used very small plastic containers to mate European *Aricia* species, provided outdoor light was used combined with good ventilation. K. Shurian (pers. comm.) required hand manipulation to mate *Polyommatus* (*Argodiatis*) species. J. Thomas (pers. comm.) was finally successful inducing mating in *Maculinea* (considered congeneric with *Glaucopsyche* by some authors) when he released adults in outdoor walk-in screen houses. This last result prompted our design of the tent cages for year 2000.

The key factors to induce mating of Palos Verdes blue butterfly are temperatures of 18–25 °C under full sunlight. These factors critically impinge on cage design because high temperatures of insolation must be avoided. Ventilation thus becomes a factor, and care must be taken to maintain high humidity. A final factor for success is aging and feeding males for at least one day prior to mating attempts. Females are immediately competent to mate on eclosion, and we have an impression that females become increasingly reluctant to mate with age.

Although we had observed mating in the gallon cages in our earliest work from 1996, results were variable. The construction of tent cages in 2000 provided an apparent ideal environment for mating. After we fabricated two prototype units the mating problem was immediately solved. When the first set of adults was introduced, matings occurred within minutes, a phenomenon we never noted in the gallon or other small cages.

Oviposition and the egg stage

Eggs are laid singly, usually on the foodplant flower buds and developing seedpods, secondarily on young stems and leaves. We have only limited data for average egg production per female because most rearing was performed using several mating pairs that were not individually segregated. From counts made, however, we observed a maximum of 187 eggs per female with many females yielding no eggs or a few sterile eggs. The latter cases were clearly the result of mating failure, a commonplace occurrence in our work with small cages. Sterile eggs were revealed by collapse of the egg between 5–8 days after laying. Eggs normally hatch in 8–10 days under ambient March temperatures.

During the 2002 breeding cycle, 14 "gallon" cages, each with two pair of adults, yielded none to about 60 eggs per cage, with a total of 317 eggs from 14 cages for an average of 11 per breeding pair (Table 1). The egg counts are approximate minimum values because of the difficulty in making accurate counts with the dense plant material present.

The Palos Verdes blue butterfly uses two foodplants on the Palos Verdes peninsula, deerweed (*Lotus scoparius*) and rattlepod (*Astragalus tricopodus*

Table 1. Number of eggs laid, larvae and pupae recovered from 12 "gallon" cages each with two pairs of adults. Two foodplants employed.

Cage	foodplant	# eggs	larvae recovered	pupae
1	*Astragalus*	none		
2	*Astragalus*	none		
3	*Astragalus*	none		
4	*Astragalus*	40+	15	none
5	*Astragalus*	20+	10	none
6	*Lotus*	50+	25	3
7	*Astragalus*	60+	20	6
8	*Lotus*	40+	25	none
9	*Lotus*	50+	15	none
10	*Lotus*	50+	30	19
11	*Lotus*	none		
12	*Lotus*	none		
13	*Lotus*	3	2	1
14	*Astragalus*	4	4	2
	Estimated total	317+	146	28

lonchus). Both were offered to the adults confined in the gallon cages (Table 1). There were no choice options in 2002, but egg productivity was similar on the two resources: 124 eggs on seven rattlepods and 193 eggs on seven deerweeds.

During earlier work we found egg counts per individual female varied widely from none (mating failure) to 187 per female. In 2000 we recovered a minimum of 500 eggs from 17 females in a walk-in screen house over an 18 day period.

Larvae

There are four larval instars. The first are translucent off-white color, cylindrical, and bear many black, long setae. At this stage the larvae are very fragile and can only be moved using fine camel hair brushes or "Q-tips." In the laboratory these neonates often move from their birthplace, leave the foodplant (even if it is robust and healthy appearing), and are subject to loss. The reason for moving is unclear; while neonates occasionally leave apparently healthy plants, they almost always leave plants with aphid infestations.

Among larvae, neonate/first instar loss was high in gallon cages kept in the laboratory. Aside from the losses associated with plant condition (aphids, wilt), a test was made in year 1999. A set of 44 fertile eggs was transferred to cups with a few fresh flower buds (deerweed) just prior to hatching. All hatched, but only 20% (9/44) survived to second instar. In a parallel trial, 35 fertile eggs were transferred to buds placed on artificial diet. All neonates perished by drowning, either by wandering onto the wet diet surface or from condensate. To what extent handling itself was responsible is not known, but the effect of handling is likely not trivial. This experiment ruled out the use of artificial diet for neonate larvae, and confirmed the observation of high mortality during the first instar.

Later (second and third) instars were usually lost to apparent disease, but at a far lower rate. During 2002, disease losses were from a microsporidium and possibly a virus, discussed below. Fewer then a dozen larvae were lost over the years showing symptoms of Bt. Last instar larvae were rarely lost, with fungal infection being the the most common cause.

In our earliest attempts at rearing the necessity of individual confinement was implemented to avoid cannibalism. Initially third instar larvae were isolated in cups and fed pieces of foodplant. Because it was necessary to replace the material daily, we switched to artificial diet for these later instar larvae.

Artificial diet

The diet designed for larval growth is given in the appendix. Rearing on artificial diet in individual containers eliminates cannibalism, provides broad-spectrum antibiotics that virtually eliminate bacterial infection, and prevents losses from predation and parasitoids. Diet feeding was not without problems; including fungal growth on frass and, during 2002, refusal of many individuals to feed. The first necessitated frass removal every few days. The second necessitated adding fresh foodplant to the containers.

We would use the diets for early instars, but in addition to neonate drowning, second instars usually refused to feed. We found that when transferring second instars on foodplant pieces (such as a flower bud), the larvae would not accept the diet until achieving at least late third instar.

The reason that most of the 2002 larval population refused the diet is unclear. Diet with the same components had been almost universally accepted before 2002. Oddly, the green hairstreak (*Callophrys affinis perplexa)* controls we reared in parallel did feed on diet that they had in all earlier trials refused. We cannot explain the phenomenon and did not have the time to experiment.

The use of artificial diet in individual containers provided antibiotic and antifungal compounds that likely had a salutary effect. Green fungal growths were mostly confined to frass, never on the surface of the diet. When frass was removed, no residual fungal growth occurred.

However, fungal growths were a problem on some of the larvae themselves. These infections were associated with the ninth segment honey-gland and were usually fatal. Fungal growths were associated with high humidity in the capped containers (the small air holes we punched did not significantly reduce container humidity) and the secretion of honeydew by some individuals.

Most larvae recovered from the tent cages were attended by Argentine ants *(Linepithema humile)*. Indeed the presence of ants was our visual clue to locate larvae at low density in the tents. When transferred into cups, the ants usually were introduced with the larvae. Because of their strong fidelity this was a byproduct of the transfer. For

larvae with attendant ants, no fungal growths were recorded, which we attributed to the continuous removal of honeydew by the ants.

Pupation

Pupation in nature takes place in the loose duff and micro-crevasses at the base of foodplants. On *Astragalus* plants, pupation sometimes occurs within seedpods. A loose girdle of a few silk threads binds the pupa to substrate. Less than 1% failure of pupation has been observed (none in 2002), usually because the larval skin could not completely shed.

During the 48-hour prepupal transition the larva is immobile while preparing for ecdysis. This is a period of extreme vulnerability to attack by predators (see below). However, under laboratory conditions predators are excluded.

Following pupation, specimens are left to harden for a week. Then the pupae are removed from cups, cleaned by washing in a 5% tween 80 solution, dipped in a 10% bleach solution, washed in distilled water, and placed on a tissue paper pad in a clean cup. After one to two months the cups are placed into plastic shoeboxes over a layer of sterile pumice stone. The boxes are placed into a refrigerator at ~4 °C. The pumice is soaked with distilled water monthly to maintain humidity.

The 2002 pupae were weighed in August with the lightest weighing 60 mg. Because all pupae have a certain likelihood of remaining in diapause, all will be set out for rearing during the 2003 season. Multiple year diapause is a common strategy of insects in unpredictable climates (Scott 1986), and Palos Verdes blue butterfly is no exception.

Problems Encountered

Aberrant Adults

Two classes of morphological anomalies were observed. The first involved defective legs. Four males and three females (4.1%) had truncated or missing tarsi. Because butterfly legs are rarely inspected either in collections, and even less often in nature, comparison of frequency of these defects to natural populations is not possible.

There were two wing pattern aberrations: 1) greatly reduced (N=11) or absent (N=17) underside secondary macules (Fig. 7) and 2) exaggerated postmedial underside macules (N= 3, Fig. 8). Frequency of both aberrant forms is very low in nature and collections across all populations of the species. It is noteworthy that both leg anomalies and wing aberrations were significantly clustered in the set of adults emerging during the first three days of eclosion (15/32 = 0.47 versus 12/135 = 0.09). We believe this is indicative of some thermal shock associated with premature ontological stages in some pupae when they were removed from refrigeration. Although there are no supporting data, the pattern implies the anomalies were developmental and not genetic.

Diseases

Lepidoptera are susceptible to a wide variety of infective diseases (Boucias & Pendland 2001). Most knowledge of diseases is a consequence of economic importance for potential specific pest control. Demographics are unquestionably affected by all disease organisms that in turn may have profound impacts on density dependent population regulation and adaptive processes. Disease organisms may indeed account for some of the order of magnitude differences periodically seen in densities of adjacent populations of species where no visible resource variation is apparent. Although not tested, the hypothesis is plausible and offers one explanation of why Palos Verdes blue butterflies are sparse by comparison with nearby southern blue butterfly populations (*Glaucopsyche lygdamus australis*).

The high density monoculture of captive breeding programs provides a high risk environment for disease. Disease control is a key management factor, with all categories of infective agents likely to play a role. Bacteria, virus, fungi, nematodes, and microsporidia have all affected lepidoptera breeding programs (Tanada & Kaya 1993).

The sporogenic bacterium *Bacillus thurengensis* (Bt) has proven a potent and widespread pathogen. Although observed in prior years, during 2002 we found no larval death from apparent Bt infection. Symptoms are cessation of feeding followed by sudden eversion of the hind gut through the rectum and almost immediate death. Bt symptoms and etiology are well known. The bacterium is apparently ubiquitous with a variety of genotypes occurring in nature with variable infectivity (Tanada & Kaya 1993). Under natural conditions epizootics are uncommon, but do occur in confined breeding.

Bt infestations may become endemic in breeding colonies with sublethal infections common. The use of antibiotics in defined diets usually maintains control, although the possibility of resistant strains arising is always present. Many other potential bacterial pathogens are likely as well.

All four major groups of viruses are known lepidopterous pathogens. Most virus usually cause rapid death in larvae terminating with a very characteristic "wilt" (Hunter-Fujita et al. 1998). Recently, Reoviruses (CPV) have been shown with serious chronic effects on insect breeding as they can be maternally transmitted (Hunter-Fujita et al. 1998). We have never observed apparent losses from classic "wilt" disintegration.

We experienced an unusual infection in a few second and third instar larvae exhibiting completely distended prothoracic segments (Fig. 9). The affected individuals ceased eating. This etiology was only seen in 2002. Of 28 noted, 20 died and 8 recovered to continue normal development. The symptom appeared only with plant-fed individuals, none with diet-raised stocks. The disease is noteworthy because some recovery occurred. The causal agent was probably a microsporidian (see Boucias & Pendland 2001). Microsporidian *Nosema* species have become endemic in pink bollworm laboratory stock. These are difficult to control, can contribute to reduced fertility in females, and are transovarian transmitted.

Besides the fungal infection noted on the larval honey gland, entomophagous fungi known from other lepidoptera have potential deleterious effects. We have not detected these. Nematodes constitute the last major potential pathogens. We have no evidence of nematode presence in our stock.

Practical control of all the above pathogens relies on cleanliness and frequent disinfection in the laboratory and use of biocide chemicals. Thus the use of defined diet, which is virtually sterile and contains antibiotics and fungicides, offers some protection in high density cultures. The apparent freedom of pathogenicity during the outdoor tent breeding suggests possible protection as a result of low density in an open complex ecosystem.

Parasitoids

Although potential parasitoids that attack all life stages are doubtless present, none have been found during any facet of the breeding program. Given exposure in the tent cages, it is particularly surprising that trichogrammid wasps have not been recovered from eggs. In spite of the tents providing access to most potential parasitoids, none have been observed.

Predators

Two predators have killed individuals in the tent cages. Several species of spiders construct webs in the tents that have trapped adults (Fig. 10). Others likely prey on larvae, although direct attack has never been observed. Small larvae do disappear. Care must taken to remove all spiders from the laboratory, where predation by spiders is possible. We have also documented predation by yellowjackets on adult butterflies (Lipman et al. 1999), but this predator is adequately excluded by the tent and gallon cages.

The most serious predation has been from the abundant European earwig, *Forficula auricularia*. The earwig is one of the most common ground dwelling insects on the site. The 2001 rearing program was devastated by earwigs as a consequence of permitting larvae to pupate in the tent cages. Table 2 gives the results of egg production, observed larvae, pupae recovered and approximate earwig density from the cages. The earwig problem was unrealized until after pupa recovery when the significant correlation became clear. The density of earwigs better explained the number of pupae recovered on the ground in the cage than any other factor. Later tests, placing mature (hardened) pupae with earwigs did not result in predation. We hypothesize that heavy predation took place during the 48-hour period of prepupal quiescence when the new pupa exoskeleton is thin.

Costs

Breeding results from 1995 to 1998 were poor, but increased effort yielded 627 pupae in 1999, 968 in 2000, with a setback to 299 in 2001 and 168 in 2002. The captive breeding program has improved and unlimited captive rearing seems attainable, given no unforeseen consequences. In 1999 the costs in time and material was about $15,000, a cost per pupa (625) of $25. The costs were about the same in 2000, with $25,000 required to produce about 1,000 pupae. In both 2001 and 2002 costs

Table 2. Pupa recovery from the nine tent cages used for captive propagation of the Palos Verdes blue butterfly at DFSP, 2001. Number of breeding adults placed in cage, foodplant species enclosed, cage bottom, estimated egg and larval density, pupae recovered, and numbers of earwigs (*Forficula auricularia*) noted at time of recovery.

Cage No.	No. Adults*	Food Plant	Ground Cover	Oviposition Dates/ N	Larvae N & notes	Pupae N	*Forticula*
1	77	L	GC	3/17-4/20 >>100	>>100 noted	6, base of FP	>100
2	71	L	GC	3/17-4/20 ca 100	ca 100 noted	5, base of FP	>100
3	61	A	B	3/17-3/25	Few late instars Aphid defoliated	12, base w 12 in A. pods	>100
4	62	L	GC	3/17-4/5 few	Few early & late instars	none	>>100 highest
5	83	L	B	3/20-4/10 >300	Many, 40 late instars 4/15	24	~100
6	92	L	B	4/1-4/17 >300	Many all instars	108	6
7	81	L	B	3/21-4/15 >100	Many all instars	68	>25
8	66	A	B	3/21-4/1 few	None seen, aphid defol.	5, w 2 in>50 A. pods	
9	80	L	GC	3/21-4/12 >>100	Few noted after 4/15	71	>25

*25 were variously lost during handling
Foodplant: L = *Lotus scoparius*, A = *Astragalus tricopodus*
Ground Cover: B = bare, GC = ground cloth of 4 mil black plastic

increased to about $50 and $100 per pupa respectively. Given that commercial rearing of lepidoptera for biological control and butterfly house display programs is on the order of $0.07 to $3.00 (depending on size and quantity), there is ample room to reduce costs. However, it must be recognized that the Palos Verdes blue butterfly is a diapausing species so high labor is required for short periods. This life history constraint does not provide for an efficient economy of scale. Lastly, both the facility and methodology are not yet optimal.

Conclusions

Observations to date provide insights that may be conveniently considered as key factor analyses under laboratory conditions. Both fecundity (number of eggs produced per female) and fertility (frequency of fertile eggs) varied enormously. Although few individual females were scored, fecundity varied from none (mostly copulation failure) to nearly 200 eggs. Fertility was usually 100%, discounting those females (see Table 1) who laid only a few sterile (collapsed) eggs or no eggs at all. The cause of infertility is assumed a failure of mating. Whether mating failure was intrinsic (genetic) or environmental is not known, although the latter is highly likely given the general mating success always noted in tent cages.

We conclude the following:

1. In comparison with three other lycaenid butterflies we have reared (Mattoni 1988), the Palos Verdes blue butterfly has been the most difficult.
2. Mating and rearing can be conducted effectively in outdoor tent cages. When late larval instar larvae are seen, they should be transferred to small cups on diet. This combined approach has so far provided the best results for mass rearing.
3. For special cases where small (e.g., gallon) cages are used, females should be permitted to oviposit for short periods of no more than 2–3 days. The cages should then be placed open, or partially

screened, in a protected outdoor location to avoid aphid infestation. Larvae should be permitted to mature within the enclosed cages. When near pupation, the larvae should be transferred to individual cups.

4. No evidence of parasitoid impact was found, even using the outdoor tent cages. Disease may or may not be an issue. The key factor limiting productivity appears to be providing an optimal environment for foodplant maintenance.

5. A pupation medium should be developed to permit ecdysis without loss to predators (earwigs) in both tents and small cages. Efforts to date indicate earwig predation is the major cause of loss if larvae are allowed to pupate outdoors.

6. Field collected females and males should be introduced into the breeding system to minimize loss of rare alleles.

7. Genetic studies would be appropriate to determine the extent of inbreeding both in the wild and captive populations. Comparative adult densities in nearby southern blue populations indicate that foodplant density is not limiting for the Palos Verdes blue butterfly.

8. Emphasis of the program must be on the capability of producing large numbers of offspring.

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APPENDIX: Lycaenid Synthetic Diet (Mattoni)

200 g dried green lentils
800 ml distilled water

Place in one liter stainless beaker.
Bring to boil and leave one hour.

Add in order:
9 g bacto-agar
25 g wheat germ
5 g bacto yeast extract
5 g Wesson salt mix
10 g cellulose flour (Solka floc or equiv.)
5 g sucrose
2 g ascorbic acid
2 g potassium sorbate
2 g methyl paraben
0.8 choline chloride
0.25 g B-sitosterol
0.25 g. chlortetracycline
0.25 g. 50% procaine penicillin
0.4 ml linseed oil (raw)

Heat mixture in boiling water bath (or double boiler on hotplate) until temperature reaches 85–90 °C, stirring occasionally.

Place mixture directly over low heat (flame or electric element) stirring constantly until mixture just comes to boil (necessary to dissolve agar).

Cool to about 80 °C.

CAREFULLY pour about 1/3 into (Waring) blender, blend for few seconds, after initial splashing, continue pouring remainder into blender until all well blended (about 30 seconds).

Dispense into containers (we use automatic pipette to dispense 5 ml aliquots into 1 oz creamers set in trays for easy handling), immediately cover with clean paper towels. Refrigerate when set (about 15 minutes), tightly enclosing trays in clean plastic bags. Can be stored under refrigeration for 60+ days.

Alternatives

1. May substitute baby lima beans or other beans for lentils.

2. May substitute complete defined vitamin mixes, or multivitamin tablets for yeast extract.